Computer Assisted Proficiency Testing in an HLA Laboratory

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ABSTRACT

The South-Eastern Organ Procurement Foundation administers a proficiency test for all of its HLA typing laboratories. This quarterly test is graded based upon consensus of values supplied by the participants. An overview of the test and the use of a computer program to aid in scoring is presented with results from recent experience.

INTRODUCTION

A major component of the algorithm for allocating human organs for transplantation is Human Leukocyte Antigens (HLA) typing. One of the oldest professional groups in transplantation, the South-Eastern Organ Procurement Foundation (SEOPF), has long been a leader in stressing the importance of antigen matching tissue as a significant factor in successful transplantations. Currently, HLA A, B, DR typing and donor/recipient crossmatch are routinely used in choosing appropriate recipients for all kidney transplants in the United States. The successful use of HLA typing in the sharing process requires proficiency testing among the HLA laboratories to help ensure quality control of this important and vital task. This paper presents an overview of the proficiency test currently coordinated by SEOPF.

HISTORY

As early as 1969, SEOPF had the rather ambitious goal of sharing organs based on match. At this time, when reagents and techniques were primitive, SEOPF provided a means of proficiency testing by conducting workshops and cell exchanges between member laboratories to evaluate various techniques, reagents and antisera. These "wet" workshops (laboratory personnel meeting at a central location for actual testing of samples) were used to ascertain how well the laboratories could agree on antigen assignments and to decide upon procedures which would allow exchange of data for regional organ sharing. As a result of these early workshops, it was initially decided to have all SEOPF laboratories use a common source for several reagents, including antisera and complement. As reagents and antisera improved, some of these requirements proved to be unnecessary, but periodic workshops to assess laboratory proficiency continued.

Over the next seven years, SEOPF membership grew to the point that it became impractical for all laboratories to meet and test at one site for "wet" workshops. Therefore, in 1976, the present proficiency testing system emerged. This system requires specimens to be sent quarterly via express carrier to each laboratory for testing. The results are tabulated by computer, reviewed, and discussed afterwards at annual "dry" workshops. The meetings also provide continuing education and a forum for discussion of problems and techniques.

Excellent correlation between laboratories in antigen assignments and crossmatching has been reported [2] from these proficiency tests. In 1981, a formal method of grading was recommended by the SEOPF Histocompatibility Committee. Grading of the laboratory's ability to correctly identify HLA-A and B locus antigens in the proficiency testing program was begun, and soon after, grading of a crossmatch test was added. Currently, HLA-A, B, DR typing and crossmatching are graded on a "pass" or "fail" status.

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In addition to the proficiency testing program, SEOPF assesses the quality of laboratories through a variety of methods, including laboratory inspection (SEOPF accepts American Society for Histocompatibility and Immunogenetics (ASHI) certification in lieu of a separate SEOPF inspection), examination of qualifications of laboratory directors and supervisors, annual recertification, evaluation of HLA discrepancies between institutions sharing organs, and a system of technical advisors and reference laboratories which are available to any subscriber laboratory needing or requesting assistance. The net result of these activities is a quality assurance program which is the most comprehensive in the country. Certification by SEOPF assures a minimum standard of excellence, the goal of which is to expedite the sharing of organs by minimizing the risks of obtaining different typing or crossmatch results at a receiving center.

The only other cell exchange programs in histocompatibility are: 1) an international cell exchange administered by the University of Los Angeles (UCLA), and 2) the ASHI/CAP survey, a joint venture ASHI and the College of American Pathologists (CAP). Although both programs use computer analysis for assessment of performance, results are mailed in and reports are returned by mail. The UCLA exchange is not really a proficiency test but a program to provide laboratories with rare and unusual cells and allow them to compare their results with other laboratories. The ASHI/CAP program is a graded proficiency test, but there are no penalties for failures nor provisions for technical assistance.

DESCRIPTION AND PARTICIPATION

All SEOPF member laboratories are required to participate in the proficiency testing program as a condition of their membership in SEOPF. Non-member laboratories may also participate in the testing. An annual fee covers the expenses incurred by the program. SEOPF laboratories are also required to send one or more technologists to the annual SEOPF Histocompatibility testing workshop.

Participating laboratories test the samples using routine procedures and reagents, and cross-match using the Amos modified technique. Assignment of HLA-A, B, DR locus antigens and crossmatch assignment by each laboratory is analyzed. Laboratories are apprised of their current performance after each exchange. Each laboratory must rotate the proficiency test among all technologists qualified to take emergency call for donor typing.

Responsibility for sending the proficiency testing specimens quarterly is rotated among four contract laboratories in each of two regions. The sending laboratory notifies each participant by letter when to expect the materials for testing. Address corrections are requested at this time to assure timely arrival of all shipments. Procedures for selection, preparation and shipment of cells are contained in a manual sent to the responsible laboratory prior to the sendout.

The sending laboratory selects four donors for each proficiency testing program. The HLA antigens of the donors selected are generally well characterized and, when possible, genotypes are available. Care is taken to provide a variety of rare and common antigens with a mixture of racial representation. Five to seven ml of anticoagulated (Heparin) blood is provided to each laboratory for testing. The provision of blood samples rather than a lymphocyte preparation is a unique feature of the SEOPF proficiency testing program which assures a better test of the laboratory's complete specimen handling and testing system. This has resulted in a superior program with fewer technical failures. Four antisera which react with one or more of the donor samples is also shipped with the cells for crossmatch testing. The sending laboratory is responsible for providing a viable sample for testing.

COMPUTER OPERATION

The proficiency testing data is entered by each laboratory via local terminal into the SEOPF computer system. Current computer equipment

consists of an IBM RS/6000 model 320 with 16 megabytes of memory and one gigabyte of disk storage. Communications is provided through four 2400 baud modems. The system is located at the SEOPF office in Richmond, Virginia.

The proficiency test software is written in ANSI standard C. The software system is entirely menu driven with appropriate on-line help. The functions provided at the top level are:

Print HLA antigen instructions
Enter HLA antigen assignments
Print crossmatch instructions
Enter crossmatch results
Print HLA antigen report

At the beginning of each test period, the software is activated to allow data entry for one week. Subscriber laboratories connect to the system using terminal facilities with modems. Laboratories who do not have terminal facilities available FAX their results to the Proficiency Testing Contractor for entry into the system. Regardless of the method of access, each user is responsible for the validity of their own data. Entry of HLA A, B and DR locus antigens is required by the testing software. The antigens are entered in numerical order. Splits are entered when identified. In addition, BW4, BW6, and percentage viability for T and B cells are entered as appropriate. The initials of the responsible technician are requested. Crossmatch data is entered for each cell/serum crossmatch as "+" for positive / incompatible, "-" for negative / compatible, or "0" for not tested / unknown.

Once the data entry period is complete, analysis programs are run to evaluate the results. Assignment of antigens is determined by a consensus of 75% of the reporting laboratories. If an antigen does not reach consensus, that particular antigen is not graded. A list of current specificities which must be identified as a minimum is revised and updated periodically by the SEOPF Histo-compatibility Committee to reflect improvement in reagents or identification of new specificities. The current list of required antigens is available on-line to all users. More than four major antigens being misidentified on any 12 month period constitutes unsatisfactory performance.

Consensus for crossmatch is determined by agreement of at least 85% of participants. If crossmatches do not reach consensus, that particular cell/serum combination is not graded. Acceptable lab performance is to have no more than four crossmatch reactions different from consensus per twelve month period.

A portion of the computer antigen report for a single cell is shown in Table 1. The crossmatch report is shown in Table 2. Cumulative proficiency test results are tabulated by computer and made available to the laboratory directors following each quarterly test. A summary report of the results and grades is mailed to each participating lab. The Proficiency Testing Subcommittee reviews each exchange and makes recommendations to the SEOPF Histocompatibility Committee for any action needed.

A laboratory which fails any part of the proficiency test is offered technical assistance. SEOPF member laboratories who fail the proficiency test must correct their deficiencies. They are required to either 1) have a site SEOPF technical advisor visit, 2) visit a reference laboratory or 3) perform successfully in an "enhanced" proficiency testing. An enhanced proficiency test involves receiving an extra shipment of cells during the next quarter to replace an exchange in which they performed poorly. Continued failure in the cell exchange can result in loss of SEOPF membership.

Corrective procedures for SEOPF member laboratories not in compliance with the minimum standards of the proficiency testing program includes the right of the participating laboratory to contest all results through the Proficiency Testing Subcommittee. Non-member institutions are also notified of any failures and also may elect to receive assistance. Data on consensus differences of non-

Table 1
HLA Antigen Report

	A1	A2	B1	B2	B4	B6	C1	C3	DR	DR	DR 52	DR 53	DQ	DQ	%VIAB TC	%VIAB BC	MAJ AB	MISS DR
Consensus	3	23		35		6	3	4	11		52							
Major AGN	3	23		35					5									
LAB 1	3	23		35		6	3	4	6	11	52			3	99	99	0	0
LAB 2	3	23		35		6	3	4	11	13	52			3	98	98	0	0
LAB 3	2	23	35			6	3	4	11	13	52			7	98		1	0
LAB 4	3	23	35			6	4		11		52		3		99	95	0	0
LAB 5	3	24		35		6	3	4	6	11	52			7			1	0
LAB 6	3	23	35			6	3	4	11	13	52		7		85	70	0	0
etc.																		

Table 2
Crossmatch Report

	CELL 213 SERUM				CELL 214 SERUM				CELL 215 SERUM				CELL 216 SERUM				MISS
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
CONS	+	-	-	+	-	+	0	-	-	+	0	-	-	-	+	-	
LAB 1	+	-	-	+	-	+	-	-	-	+	-	-	-	-	+	-	0
LAB 2	+	-	-	+	-	+	+	-	-	+	+	-	-	-	+	-	0
LAB 3	+	-	-	+	-	+	+	-	-	-	-	-	-	-	+	-	1
LAB 4	+	+	-	+	-	+	+	-	-	+	+	-	-	-	+	-	1
LAB 5	+	0	0	+	0	+	+	0	0	+	0	0	0	0	+	0	9
LAB 6	+	+	-	+	-	+	-	+	-	+	-	-	-	-	+	-	2
LAB 7	+	-	-	+	-	+	+	-	-	+	+	-	-	-	+	-	0
etc.																	

major antigens is collected for information purposes and reported periodically to the Histocompatibility Committee.

SEOPF uses the proficiency testing program upon occasion to analyze other quality assurance questions. Regional crossmatch tray analysis and laboratory Panel Reactive Antibody (PRA) assignments have been studied. However, any other uses of the specimens sent are secondary to the primary role of proficiency testing. ABO (red blood cell) antigen testing is not provided by SEOPF. However, any SEOPF laboratory reporting ABO results must participate in an approved red blood cell proficiency testing program.

CONCLUSIONS

The overall results of the testing laboratories has been excellent. HLA agreement has been above 93% for the past three years. Crossmatch grading has proved to be more difficult. Consensus cutoff has been set at 85% to try to alleviate some of the problems. Whenever consensus is reached, agreement is better than 98%. However,

approximately 17% of the cell/serum combinations have failed to reach consensus.

The overall performance of the laboratories is periodically reviewed to evaluate the testing procedures. Results of the tests are kept for at least five years and are available upon request to state and federal regulating agencies for clinical laboratories.

Whereas the testing procedures are stable, the software supporting the system as undergone major changes over the past several years. Prior to 1993, the system was written in FORTRAN on a DEC VAX cluster using machine dependent code. Today, the system is written entirely in ANSI standard C resulting in ease of maintenance as well as portability to other computers. The next version of the system will focus on the user interface with the capability full screen data entry. Microcomputer versions of the data entry programs are envisioned allowing the user to enter data locally to be transmitted as a batch. This will result in reduced operating costs for communications as well as provide a more user friendly environment.

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